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Evaluation of Mutagenic Characteristics of Diesel Hydrocarbons

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16. ABSTRACT

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Several attempts at developing a test procedure to collect the gaseous organics behind particulate filters have failed due to inadequacy of the hydrocarbon absorbing media. This study evaluates previously exposed diesel particulate filters as a collection medium after the filters have been baked at about 1000°F to remove the particulate-bound organics.

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Evaluation of Mutagenic Characteristics of Diesel Gaseous Hydrocarbons

by

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July, 1981

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Emission Control Technology Division
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U.S. Environmental Protection Agency

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Background

The organic materials extracted from diesel particulate produce a positive mutagenic response in the Ames Salmonella test. Since a small amount of high molecular weight hydrocarbon material passes through particulate filters in a gaseous form, there has been a persistent concern that these gaseous organics may also be mutagenic.

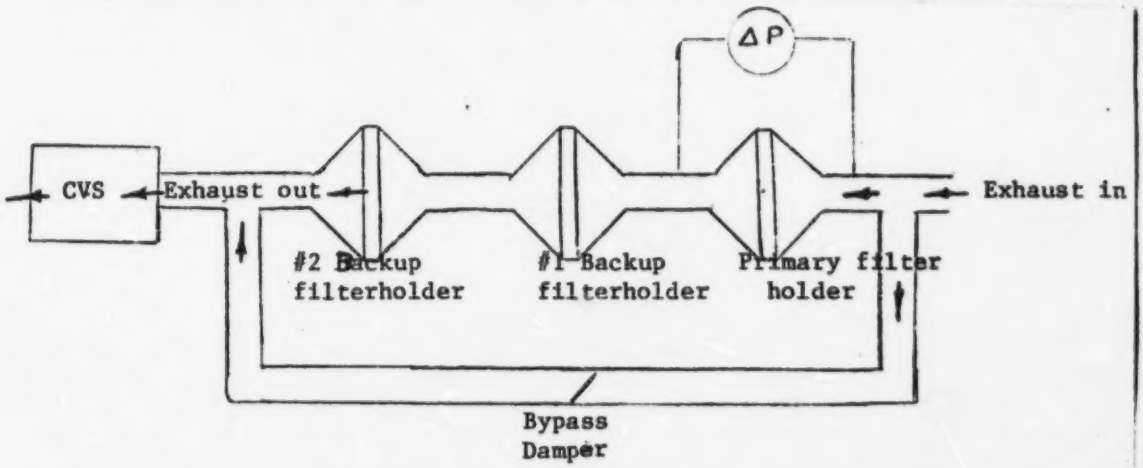
Several attempts at developing a test procedure to collect the gaseous organics behind particulate filters have failed due to inadequacy of the hydrocarbon absorbing media. This study evaluates previously exposed diesel particulate filters as a collection medium after the filters have been baked at about 1000°F to remove the particulate-bound organics.

Test Procedure

The test procedure involves collection of diesel particulate on fluorocarbon coated glass fiber filters, baking off the particle-bound hydrocarbons, and then exposing the baked filter to diesel gaseous hydrocarbons.

A. Collection System

The particulate collection system consisted of a 20" by 20" fiberglass filter commonly used to collect samples for subsequent Ames Test analysis. The filter was positioned as shown below in a fine wire mesh filter holder designated the primary filter location.



Gaseous Hydrocarbon Collection System

Diluted diesel exhaust was pulled through the filter. The bypass damper was adjusted to maintain a maximum filter temperature of 125°F. This resulted in approximately 1/3 of the flow passing through the filter and 2/3 bypassed. The exhaust gas was pulled through the filter via a constant volume sampler. The CVS was not calibrated so total flow through the filter was not measured. Therefore, revertent/mile calculations are not possible. However, filter to filter comparisons can be made. The exhaust from an Oldsmobile diesel was collected for the backup filters using the Highway Fuel Economy Test (HFET) driving schedule. The filters were removed and weighed. These weights minus the original filter weight gave the net filter loading.

The primary filter holder is followed by two identical filter holders. When the previously collected filters had been baked at 1000°F for one hour they were put into the #1 and #2 back-up filter holders. A double thickness clean filter was installed in the primary filter holder. A test sequence, FTP or HFET, was then run drawing exhaust gas through all 3 filter holders in series as shown above. The filters were removed and weighed. The difference between the post-test weights and the after-baking weight was the weight of absorbed gaseous hydrocarbons and any diesel particulate which passed through the double primary filter.

Preliminary testing using all 3 filter holders with clean filters verified a very minimal weight gain on the #1 and #2 backup filters. This weight gain was on the order of .05 grams. The actual results of this system checkout are given below:

System Checkout Test Results

Filter Position	Cycle	Before Wt. (gms)	After wt. (gms)	weight gain (gms)
#1 Backup	HFET	19.68	19.73	+.05
#2 Backup	HFET	19.55	19.61	+.06

The fact that the #2 backup filter actually had a larger weight gain than the #1 backup filter indicates that the weight gain may not be from diesel particulate but from absorption of gaseous hydrocarbons on the clean filter. Such absorbed hydrocarbons would be driven off during the baking process. The primary filter was a single filter. All subsequent tests were run with a double primary filter. With the double primary filter and with the baking procedure, it was felt that the .05 - .06 gram weight gain would not significantly affect the results of the experiment.

B. Baking System

A problem encountered with baking the filters was the readiness with which the diesel particulate oxidized. To avoid this oxidation the following system was used. The 20"x20" filter was placed on the inner circumference of a 6 inch diameter glass tube which was sealed on one end. A tight fitting glass top was installed on top of the tube. In the top was a small hole through which a stainless steel nitrogen purge line and a J type thermocouple were positioned. This container was then placed in a 24 inch diameter ceramic kiln. The purge and thermocouple lines were attached to an N_2 cylinder and a 0-2000°F J type thermocouple recorder, respectively. The oven was then heated and controlled by the operator at 1000°F for one hour. About two hours were needed to raise the oven to 1000°F, bake for one hour, and cool down the oven.

C. Testing Samples

The actual test samples were divided into five groups.

1. Group 1 - Not Baked

This group consisting of sample numbers 320, 330, and 340 was not baked. After collection, the filters were weighed and sent for extraction of organics. These filters served as a basis for determining the effectiveness of the baking procedure.

2. Group 2 - Baked not Exposed

This group, consisting of sample numbers 350, 360, and 370, was baked and sent directly for extraction. These filters compared to Group 1, showed the feasibility of driving off the extractable organics by baking. They also gave a baseline of extractable organics for the filters exposed to gaseous hydrocarbon.

3. Group 3 - Oldsmobile Results

This group, consisting of sample numbers 380, 390, 400, and 410, were the baked filters put into backup filter holders #1 and #2 for either one Federal Test Procedure (FTP) or one HFET cycle. The exhaust gas was drawn through all 3 filter holders during the complete test sequence using an Oldsmobile Diesel as the test vehicle.

4. Group 4 - Mercedes Benz Results

This group, consisting of sample numbers 420, 430, 440, and 450, is identical to group 3 but run using a Mercedes Benz as the test vehicle.

5. Group 5 - Background Air Results

This group, consisting of sample numbers 460, 470, 480 and 490, was the same as groups 3 and 4 except no test vehicle was used. Background air was drawn through the filters for the length of either an FTP or an HFET sequence. These samples were taken to verify that the extracted organics were due to the vehicle exhaust and not to ambient air hydrocarbons.

Test Results

The test result analysis will be divided into four sections covering filter weights, extractable organic, BAP, and Ames Analysis.

A. Filter Weights

The filters were weighed in a clean condition with the initial particulate loading, after baking, and after exposure to the gaseous diesel exhaust. The filter weights are given below:

Table I
Filter Weights
All Weights in Grams

	Filter No.	Fresh Filter Weight	Initial Filter Loaded Weight	Weight from Loading	Baked Filter Weight	Weight Loss from Baking	Exposed Filter Weight	Absorbed Weight Gain
Group 1	320	19.62	21.96	2.34	N/A	-	N/A	-
	330	19.56	20.69	1.13	N/A	-	N/A	-
	340	19.55	20.47	.92	N/A	-	N/A	-
Group 2	350	19.87	21.31	1.44	20.74	.57	N/A	-
	360	19.67	20.82	1.15	20.29	.53	N/A	-
	370	19.59	20.77	1.11	20.32	.38	N/A	-
Group 3	380-1F*	19.73	22.18	2.45	21.49	.69	21.62	.13
	390-2F*	19.82	20.73	.91	20.15	.58	20.35	.20
	400-1H	19.66	21.27	1.61	20.79	.48	21.05	.26
	410-2H	19.62	21.24	1.62	20.83	.41	21.04	.21
Group 4	420-1F	19.50	20.83	1.33	20.55	.28	20.69	.14
	430-2F	19.46	20.88	1.42	20.58	.30	20.69	.11
	440-1H	19.48	20.82	1.34	20.58	.27	20.73	.18
	450-2H	20.21	22.07	1.86	21.72	.35	21.94	.22
Group 5	460-1F	20.23	22.31	2.08	21.97	.34	21.98	.01
	470-2F	20.24	21.30	1.06	21.03	.27	21.06	.03
	480-1H	20.18	22.60	2.42	22.23	.37	22.31	.08
	490-2H	20.10	22.56	2.46	22.14	.42	22.02	-.12

*The number indicates the backup filter location, either #1 backup or #2 backup. The letter indicates the test sequence used during the exposure of this filter; F = FTP, H = HFET.

Graphical presentation of the filter weights is given in Figures 1-4. The weight gains compared to the 100% Dilution Air samples indicate clearly that the filters exposed to the gaseous exhaust did achieve a positive weight gain.

B. Extractable Organic Weight Analysis

The extractable organic weights are given below. Also calculated is the percentage of extractable organic to weight gain.

Table II
Extractable Organics (all weights in grams)

	Filter No.	Filter Weight Gain	Ext. Organic Wt.	% Ext/Wt. Gain
Group 1	320	-	.33005	-
Un-Baked	330	-	.14995	-
	340	-	.17259	-
Group 2	350	-	.0030	-
Baked but	360	-	.0052	-
Not Exposed	370	-	.00140	-
Group 3	380	.13	.16215	124.7%
Oldsmobile	390	.20	.04655	23.3%
	400	.26	.12038	46.3%
	410	.21	.08805	41.9%
Group 4	420	.14	.05866	41.9%
Mercedes	430	.11	.04838	43.9%
Benz	440	.18	.06837	37.9%
	450	.22	.06070	27.6%
Group 5	460	.01	.00562	56.2%
Background	470	.03	.00815	27.2%
Air	480	.08	.00660	8.25%
	490	.12	.00506	4.22%

These results are not easily understood. It appears that filter #380 did not have all of the extractable organic baked off since the extractable weight was higher than the total weight gain. Therefore, results from that filter are questionable. The percent extractable/weight-gained figures average 37.5% for the exposed filters (other than filter #380). The reason for this low percentage is probably due to desorbtion of light hydrocarbons between the time of weighing and the time of extraction even though after weighing, the filters were refrigerated at approximately 0°F to minimize this desorbtion. The percentages for the 100% Dilution Air are wide spread. However, considering the very low filter weight gains, the percentage figures for this group are not considered to be very meaningful.

The data does indicate that the baking procedure was very effective at driving off extractable organics (other than filter #380). Filter numbers 350, 360, and 370 indicate almost no extractable organic. The four filters exposed to 100% dilution air show that very little extractable organic was adsorbed from background air. These four filters will serve well as a control for comparison with other filters. Unfortunately, by baking the filters and driving off the extractable organics, no Ames analysis can be performed on these control filters. Therefore, the results discussed later are based on the assumption of no activity from the control filters. The small amount of extractable organic taken from the control filters was used on Strain TA98 and tend to confirm this assumption.

C. BAP Results

The BAP results are given below:

Table IV
BAP Results (all weights in ng.)

	Filter #	BAP (ng)
Group 1	320	1252
Unbaked	330	528
	340	312
Group 2	350	BMD
Baked	360	16
Not Exposed	370	20
Group 3	380	48
Oldsmobile	390	96
	400	BMD
	410	BMD
Group 4	420	BMD
Mercedes	430	BMD
Benz	440	BMD
	450	40
Group 5	460	BMD
Background	470	40
Air	480	360
Air	490	BMD

*BMD = Below measurable detection.

These results indicate normal diesel particulate BAP levels for filters 320, 330, and 340. The baked filters, other than filter number 480, show very little BAP. Filter #480 is considered to be a testing anomaly.

D. Ames Analysis

The Ames test is normally run using five different strains of bacteria. Due to limited extractable organic, some strains were not run. Each strain, if possible, was run both with and without metabolic activation. The analysis is done in cultura plates, and is expressed in revertants/microgram extractable organic. This result indicates the mutagenicity of the extractable organic.

The Ames results then do not take into account the different extraction rates. The actual Ames results and the dates the samples were run are given below. Also given are the revertants/filter. Since identical tests were run for each set of filters, a direct comparison of revertants/filter is possible.

Table V
Strain TA-98

		Non Activated		Activated	
Filter #		Rev./ug.ext.org. 2-27-81/3-24-81	Rev. per filter* 2-27-81/3-24-81	Rev./ug. ext.org. 2-27-81/3-24-8	Rev./Filter* 2-27-81/3-24-81
Group 1	320	14.1/13.5	4653/4455	8.1/7.0	2673/2310
Unbaked	330	10.2/11.9	1514/1784	9.0/7.8	1350/1170
	340	5.8/3.3	1001/569	9.8/7.2	1691/1243
Group 2	350	0.3	0.9	-	-
Baked	360	0.3	0.15	-	-
Not Exposed	370	1.4	1.96	-	-
Group 3	380	18.9/36.8	3065/5967	2.4/.8	389/130
Oldsmobile	390	6.4/12.5	298/582	.5/.6	23.3/27.9
	400	17.9/11.0	2155/1324	.6/.6	72.2/72.2
	410	8.5/8.0	748/704	.6/.3	52.8/26.4
Group 4	420	10.1/14.7	592/862	1.4/.6	82.1/35.2
Mercedes	430	.8/.3	38.7/14.5	.4/.1	19.4/4.84
Benz	440	28.3/18.6	1935/1272	4.1/2.5	283/171
	450	1.6/2.5	97.1/151	.4/.1	24.3/6.07
Group 5	460	0.5	2.81	-	-
Background	470	0.2	1.63	-	-
Air	480	2.0	13.2	-	-
	490	0.1	.51	-	-

* times 10^3

Table VI
Strain TA-100

		Non Activated		Activated	
Filter #		kev./ug.ext.org. 2-27-81/4-10-81	Rev. per filter* 2-27-81/4-10-81	Rev./ug.ext.org. 2-27-81/4-10-81	Rev./Filter* 2-27-81/4-10-81
Group 1	320	27.0/17.7	8911/5776	8.1/13.5	2673/4456
Unbaked	330	47.3/20.7	7093/3104	9.0/9.0	1349/1349
	340	5.9/12.2	1018/2106	6.2/6.0	1070/1035
Group 3	380	3.9/44.7	632/7248	21.9/11.7	3551/1897
Oldsmobile	390	14.8	689	-	
	400	59.1/20.5	7114/2468	4.3/4.4	518/530
	410	18.7/8.7	1646/766	6.1/3.3	537/290
Group 4	420	14.9	874	4.3	252
Mercedes	430	2.6	126	-	-
Benz	440	2.5	171	24.7	1689
	450	1.4	85	3.1	188

* times 10^3

Table VII
Strain TA-1535

		Non Activated		Activated	
	Filter #	Rev./ug.ext.org. 3-6-81	Rev./filter* 3-6-81	Rev./ug.ext.org. 3-6-81	Rev./Filter* 3-6-81
	320	0.1	33.0	0.3	99.0
Group 1	330	0.6	90.0	0.1	15.0
Unbaked	340	0.0	0.0	0.3	51.8
Group 2 Oldsmobile	380	0.8	130	0.0	0.0

* times 10^3

Table VIII
Strain TA-1537

		Non Activated		Activated	
	Filter #	Rev./ug.ext.org. 3/6/81	Rev./filter* 3/6/81	Rev./ug.ext.org. 3/6/81	Rev./Filter* 3/6/81
Group 1	320	1.9	627	3.1	1023.
Unbaked	330	2.7	405	2.5	375
	340	1.1	190	1.7	193
Group 3 Oldsmobile	380	1.7	275.	.4	64.8

* times 10^3

Table IX
Strain TA-1538

		Non Activated		Activated	
	Filter #	Rev./ug.ext.org. 3-6-81/4-10-81	Rev./filter* 3-6-81/4-10-81	Rev./ug.ext.org. 3-6-81/4-1-/81	Rev./Filter* 3-6-81/4-10-81
Group 1	320	6.3/8.3	2079/2739	10.1/7.7	3334/2541
Unbaked	330	7.3/3.4	1094/510	10.7/9.6	1604/1440
	340	2.5/2.1	431/315	2.7/2.8	465/483
Group 3	380	.6/5.5	97/892	2.5/1.6	405/259
Oldsmobile	400	2.5/1.4	301/168	.8/1.3	96/156

* times 10^3

Analysis of Ames Results

Since all strains were not run for all filters due to lack of extractable organics, the analysis is not complete. The following comments on the Ames data can be made:

1. The diesel exhaust samples do not appear to be sensitive to strains TA-1535 and TA-1537 either activated or non-activated. The results will then be based on strains TA-98, TA-100, and TA-1538.

2. The three unbaked samples 320, 330, and 340 showed wide variability in all three strains. The other samples appear to be less widely distributed.

3. The #1 backup filters have significantly higher activity than the #2 backup filters for the three strains. This is consistent with the theory that more of the gaseous hydrocarbons were removed by the #1 backup filter leaving less to be adsorbed by the #2 backup.

4. The activated samples were less mutagenic than the non-activated samples for strains TA-98 and TA-100. This indicates the presence of direct-acting mutagens. Direct acting mutagens do not require metabolic activation in order to obtain a positive response. (Indirect-acting mutagens require metabolic activation - they are metabolically converted to an active mutagenic form.) The activity was about even for strain TA-1538.

5. The activity of the control samples on strain TA-98 confirm the assumption that very little activity was due to dilution air or organics which survived the baking process.

6. The ratio of the total number of revertents from the backup filters compared to the Group 1 filters is an indication of the amount of mutagenic activity which would not have been accounted for on the primary filter. Percentages are given on the next page (Table X) for Group #3 and #4 filters compared to the average of Group #1 filters.

   These results indicate that materials that are mutagenic passed through the primary filters and were adsorbed on the backup filters. In some cases, the backup filters were more reactive than the primary filters. These Ames results indicate that results based on primary filters only may be very conservative estimates of the true activity of the diesel exhaust.

7. The FTP and HFET results showed varied behavior. The Oldsmobile data indicated higher activity during the FTP than for the HFET cycle for strain TA-98 and vice versa for strain TA-100. The Mercedes indicated exactly the opposite results with higher activity during the HFET for strain TA-98 and lower activity during the FTP for strain TA-100.

8. The activity on the Oldsmobile was greater than that noted for the Mercedes filters for both strains TA-98 and TA-100.

9. There are several problems with the data. If the experiments were rerun, several more precautions would be taken. These problems are noted below. The reason these problems were not addressed is that until the extraction and Ames results were completed the feasibility of the testing procedure was not known.

   a. Lack of extractable organic was the largest problem. More filters should have been taken to allow full Ames testing on all strains.

Table X
Ratio of Ames Test Activities; Backup Filter/Primary Filter

		Strain TA 98				Strain TA-100				Strain TA-1538			
		Non-Activated		Activated		Non-Activated		Activated		Non-Activated		Activated	
		2-27-81	3/24/81	2/27/81	3/24/81	2/27/81	4/10/81	2/27/81	4/10/81	3/6/81	4/10/81	3/6/81	4/10/81
Group 3	380	128%	263%	20.4%	6.8%	11.1%	198%	209%	83.2%	8.1%	75.1%	22.4%	17.4%
Oldsmobile	390	12.5%	25.6%	1.2%	1.5%	12.1%	-	-	-	-	-	-	-
	400	90.2%	58.4%	3.8%	3.8%	125%	67.4%	30.5%	23.2%	25.1%	14.1%	5.3%	10.5%
	410	31.3%	31.0%	2.8%	1.4%	29.0%	20.9%	31.6%	12.7%	-	-	-	-
	420	24.8%	38.0%	4.3%	1.8%	15.4%	-	14.8%	-	-	-	-	-
Group 4	430	1.6%	0.6%	1.0%	0.2%	2.2%	-	-	-	-	-	-	-
Mercedes	440	81.0%	56.0%	14.8%	8.9%	3.0%	-	99.5%	-	-	-	-	-
Benz	450	4.1%	6.6%	1.3%	0.3%	1.4%	-	11.1%	-	-	-	-	-

b. No Mercedes unbaked filters were taken and sent for analysis. Thus, the ratio of backup filter activity to primary filter activity is based on Oldsmobile primary filters.

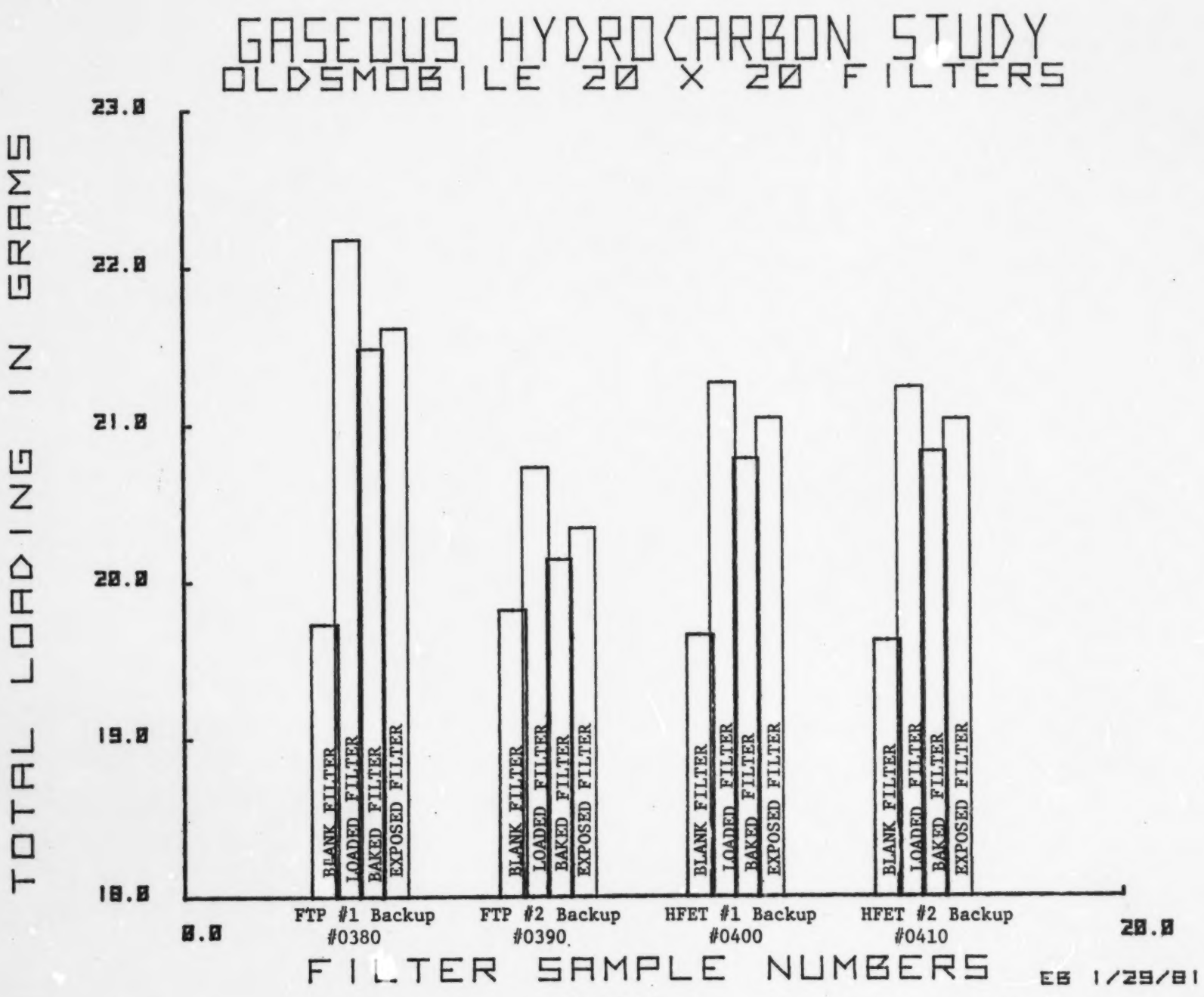
c. Unloaded filters should have been baked and inserted into filter holders #1 and #2 for an FTP and for an HFET test cycle. The filters should have then been sent for analysis.

Conclusions

1. The data indicate that gaseous materials present in diesel exhaust are mutagenic based on the Ames test results. The data also appear to indicate the presence of direct acting mutagens. Both the gas phase and particle-bound HC are more mutagenic in the Ames test without metabolic activation.

2. The assessment of the mutagenic activity of diesel exhaust using only the particulate organic may be overly conservative if significant activity is indeed present in the gas phase as these experiments indicate.

3. While this method of collection shows promise, further development work is needed to make it a routine collection procedure.


GASEOUS HYDROCARBON STUDY
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TOTAL LOADING IN GRAMS
23.0
22.0
21.0
20.0
19.0
18.0
BLANK FILTER
LOADED FILTER
BAKED FILTER
EXPOSED FILTER
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FTP #2 Backup #0390
HFET #1 Backup #0400
HFET #2 Backup #0410
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FILTER SAMPLE NUMBERS
EB 1/29/81

Figure 1

GASEOUS HYDROCARBON STUDY
MERCEDES 300D 20 X 20 FILTERS

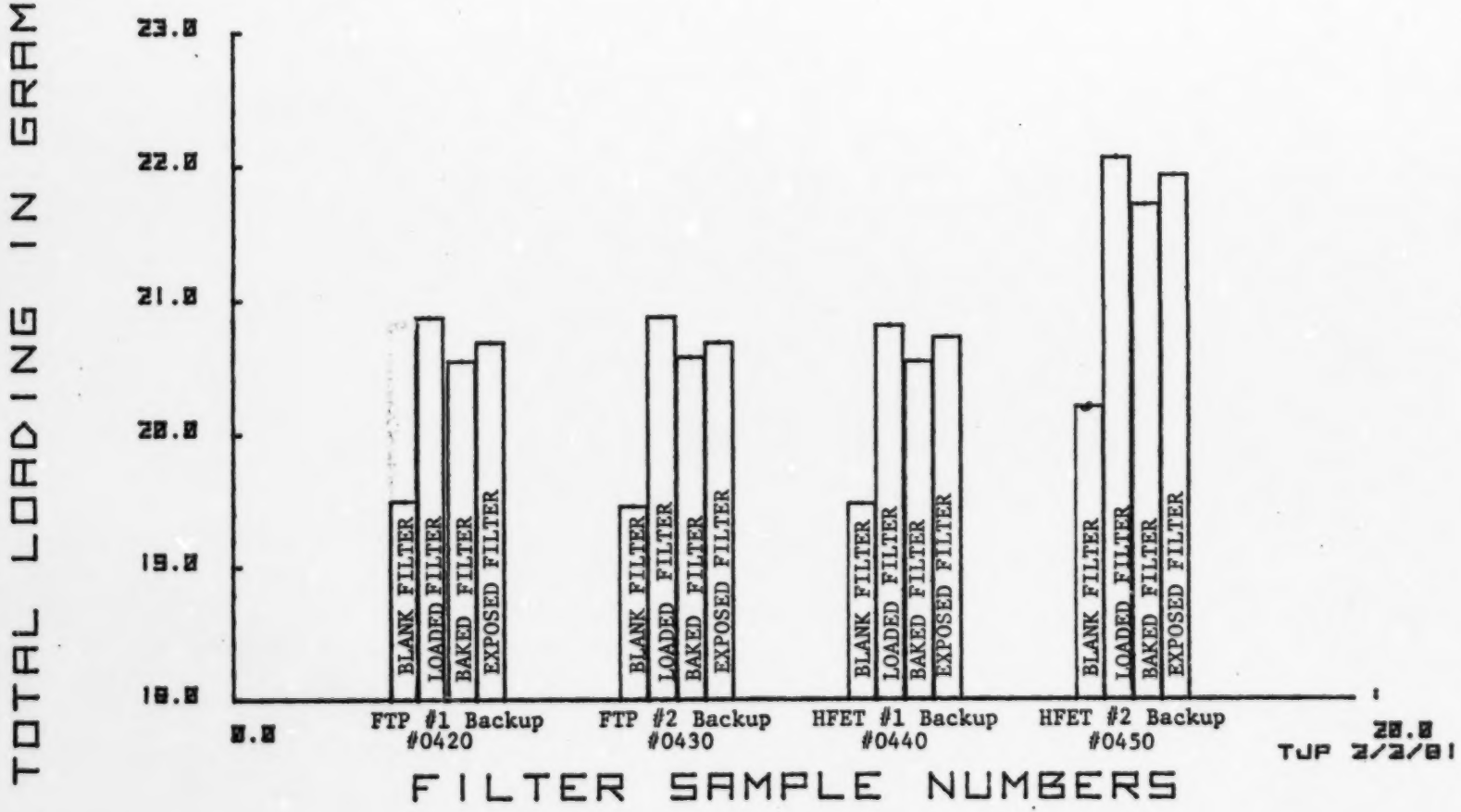
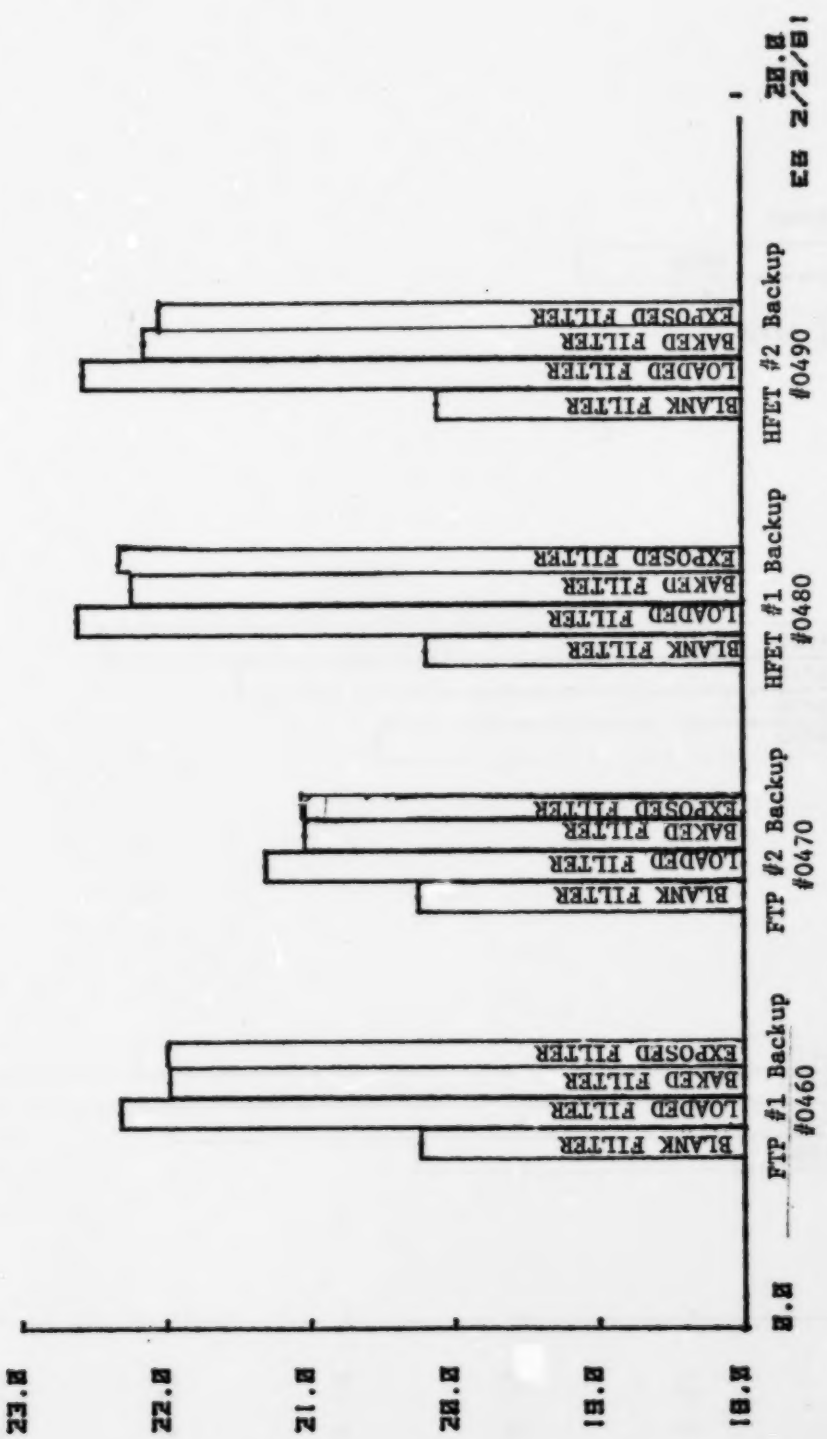


Figure 2


GASEOUS HYDROCARBON STUDY
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FTP #2 Backup #0470
HFET #1 Backup #0480
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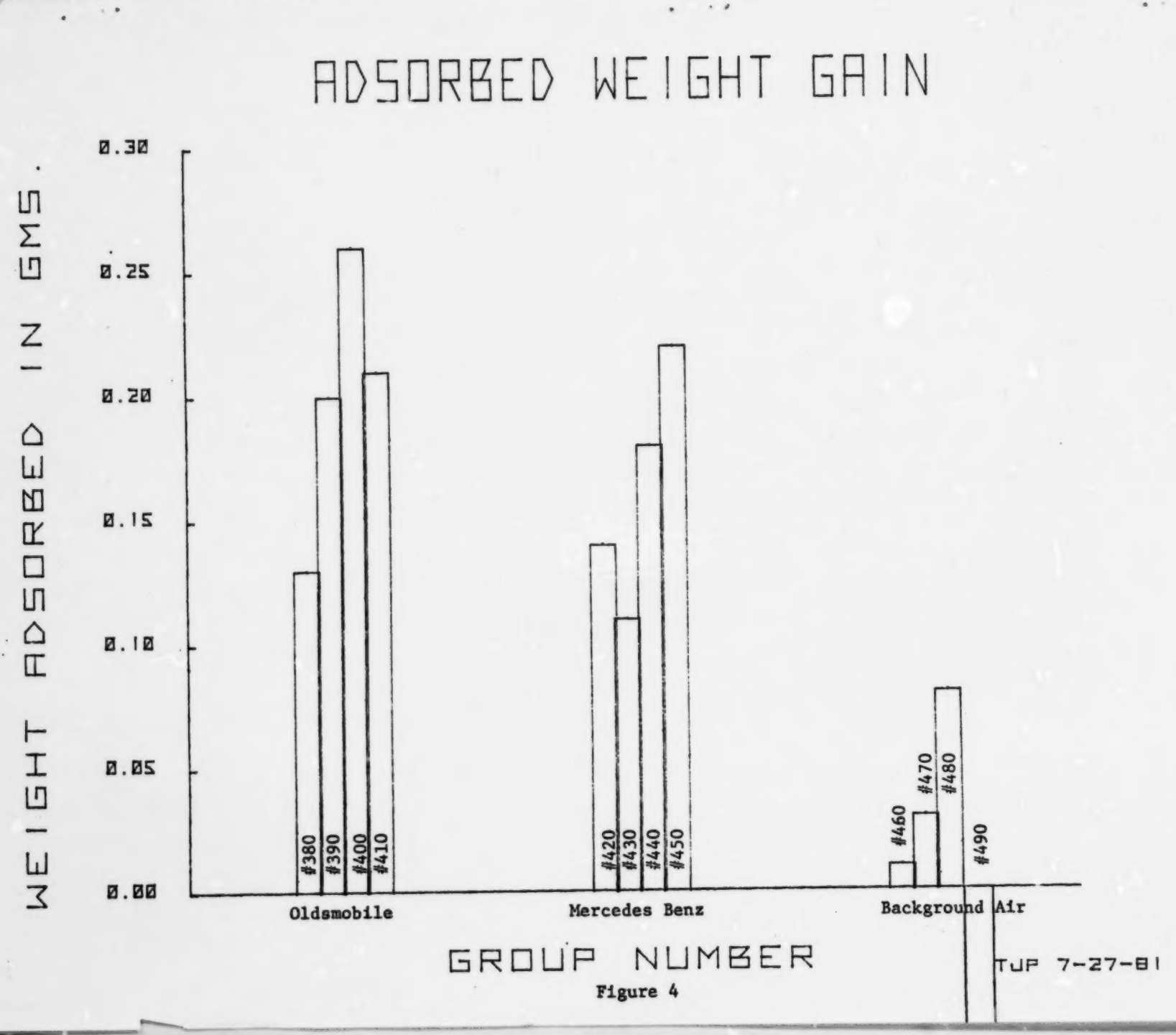
EXPOSED FILTER
FILTER SAMPLE NUMBERS

Figure 3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Mercedes Benz
Background Air
GROUP NUMBER
TJP 7-27-81

Figure 4